

NIH Biosafety Level 3 (BL3) - (Large Scale)

These questions are based on the Biosafety Level 3 (BL 3) - Large Scale section of the *NIH Guidelines for Research Involving Recombinant DNA Molecules*, March 1996 edition, Appendix K- II and K- V.

Complete this section only if your laboratory does large scale research or production using large quantities (greater than 10 liters of culture) of viable organisms containing recombinant DNA molecules.

Please circle the response that best describes the laboratory in which work with select agents will be carried out.

N.A. = not applicable. If you mark "N.A." , please provide a brief explanation below that item or on a separate page.

Appendix K-II. Good Large Scale Practice (GLSP)

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| Yes, No, N.A. | Appendix K-II-A. Institutional codes of practice shall be formulated and implemented to assure adequate control of health and safety matters. |
| Yes, No, N.A. | Appendix K-II-B. Written instructions and training of personnel shall be provided to assure that cultures of viable organisms containing recombinant DNA molecules are handled prudently and that the work place is kept clean and orderly. |
| Yes, No, N.A. | Appendix K-II-C. In the interest of good personal hygiene, facilities (e.g., hand washing sink, shower, changing room) and protective clothing (e.g., uniforms, laboratory coats) shall be provided that are appropriate for the risk of exposure to viable organisms containing recombinant DNA molecules. Eating, drinking, smoking, applying cosmetics, and mouth pipetting shall be prohibited in the work area. |
| Yes, No, N.A. | Appendix K-II-D. Cultures of viable organisms containing recombinant DNA molecules shall be handled in facilities intended to safeguard health during work with microorganisms that do not require containment. |
| Yes, No, N.A. | Appendix K-II-E. Discharges containing viable recombinant organisms shall be handled in accordance with applicable governmental environmental regulations. |
| Yes, No, N.A. | Appendix K-II-F. Addition of materials to a system, sample collection, transfer of culture fluids within/between systems, and processing of culture fluids shall be conducted in a manner that maintains employee's exposure to viable organisms containing recombinant DNA molecules at a level that does not adversely affect the health and safety of employees. |
| Yes, No, N.A. | Appendix K-II-G. The facility's emergency response plan shall include provisions for handling spills. |

Appendix K-V

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| Yes, No, N.A. | Appendix K-V-A. Spills and accidents which result in overt exposures to organisms containing recombinant DNA molecules are immediately reported to the Biological Safety Officer, Institutional Biosafety Committee, and other appropriate authorities (if applicable). Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained. |
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- Yes, No, N.A. Appendix K-V-B. Cultures of viable organisms containing recombinant DNA molecules shall be handled in a closed system (e.g., closed vessels used for the propagation and growth of cultures) or other primary containment equipment (e.g., Class III biological safety cabinet containing a centrifuge used to process culture fluids) which is designed to prevent the escape of viable organisms. Volumes less than 10 liters may be handled outside of a closed system provided all physical containment requirements specified in Appendix G-II-C, Physical Containment Levels--Biosafety Level 3, are met.
- Yes, No, N.A. Appendix K-V-C. Culture fluids (except as allowed in Appendix K-V-D) shall not be removed from a closed system or other primary containment equipment unless the viable organisms containing recombinant DNA molecules have been inactivated by a validated inactivation procedure. A validated inactivation procedure is one which has been demonstrated to be effective using the organisms that will serve as the host for propagating the recombinant DNA molecules.
- Yes, No, N.A. Appendix K-V-D. Sample collection from a closed system, the addition of materials to a closed system, and the transfer of culture fluids from one closed system to another shall be conducted in a manner which prevents the release of aerosols or contamination of exposed surfaces.
- Yes, No, N.A. Appendix K-V-E. Exhaust gases removed from a closed system or other primary containment equipment shall be treated by filters which have efficiencies equivalent to high efficiency particulate air/HEPA filters or by other equivalent procedures (e.g., incineration) to prevent the release of viable organisms containing recombinant DNA molecules to the environment.
- Yes, No, N.A. Appendix K-V-F. A closed system or other primary containment equipment that has contained viable organisms containing recombinant DNA molecules shall not be opened for maintenance or other purposes unless it has been sterilized by a validated sterilization procedure. A validated sterilization procedure is one which has been demonstrated to be effective using the organisms that will serve as the host for propagating the recombinant DNA molecules.
- Yes, No, N.A. Appendix K-V-G. A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules shall be operated so that the space above the culture level will be maintained at a pressure as low as possible, consistent with equipment design, in order to maintain the integrity of containment features.
- Yes, No, N.A. Appendix K-V-H. Rotating seals and other mechanical devices directly associated with a closed system used to contain viable organisms containing recombinant DNA molecules shall be designed to prevent leakage or shall be fully enclosed in ventilated housings that are exhausted through filters which have efficiencies equivalent to high efficiency particulate air/HEPA filters or through other equivalent treatment devices.
- Yes, No, N.A. Appendix K-V-I. A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules and other primary containment equipment used to contain operations involving viable organisms containing recombinant DNA molecules shall include monitoring or sensing devices that monitor the integrity of containment during operations.
- Yes, No, N.A. Appendix K-V-J. A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules shall be tested for integrity of the containment features using the organisms that will serve as the host for propagating the recombinant DNA molecules. Testing shall be accomplished prior to the introduction of viable organisms containing recombinant DNA molecules and following modification or replacement of essential containment features. Procedures and methods used in the testing shall be appropriate for the equipment design and for recovery and demonstration of the test organism. Records of tests and results shall be maintained on file.
- Yes, No, N.A. Appendix K-V-K. A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules shall be permanently identified. This identification shall be used in all records reflecting testing, operation, maintenance, and use of this equipment for research production activities involving viable organisms containing recombinant DNA molecules.

- Yes, No, N.A. Appendix K-V-L. The universal biosafety sign shall be posted on each closed system and primary containment equipment when used to contain viable organisms containing recombinant DNA molecules.
- Yes, No, N.A. Appendix K-V-M. Emergency plans required by Sections IV-B-2-b-(6), Institutional Biosafety Committee, and IV-B-3-c-(3), Biological Safety Officer, shall include methods and procedures for handling large losses of culture on an emergency basis.
- Yes, No, N.A. Appendix K-V-N. Closed systems and other primary containment equipment used in handling cultures of viable organisms containing recombinant DNA molecules shall be located within a controlled area which meets the following requirements:
- Yes, No, N.A. Appendix K-V-N-1. The controlled area shall have a separate entry area. The entry area shall be a double-doored space such as an air lock, anteroom, or change room that separates the controlled area from the balance of the facility.
- Yes, No, N.A. Appendix K-V-N-2. The surfaces of walls, ceilings, and floors in the controlled area shall be such as to permit ready cleaning and decontamination.
- Yes, No, N.A. Appendix K-V-N-3. Penetrations into the controlled area shall be sealed to permit liquid or vapor phase space decontamination.
- Yes, No, N.A. Appendix K-V-N-4. All utilities and service or process piping and wiring entering the controlled area shall be protected against contamination.
- Yes, No, N.A. Appendix K-V-N-5. Hand washing facilities equipped with foot, elbow, or automatically operated valves shall be located at each major work area and near each primary exit.
- Yes, No, N.A. Appendix K-V-N-6. A shower facility shall be provided. This facility shall be located in close proximity to the controlled area.
- Yes, No, N.A. Appendix K-V-N-7. The controlled area shall be designed to preclude release of culture fluids outside the controlled area in the event of an accidental spill or release from the closed systems or other primary containment equipment.
- Yes, No, N.A. Appendix K-V-N-8. The controlled area shall have a ventilation system that is capable of controlling air movement. The movement of air shall be from areas of lower contamination potential to areas of higher contamination potential. If the ventilation system provides positive pressure supply air, the system shall operate in a manner that prevents the reversal of the direction of air movement or shall be equipped with an alarm that would be actuated in the event that reversal in the direction of air movement were to occur. The exhaust air from the controlled area shall not be recirculated to other areas of the facility. The exhaust air from the controlled area may not be discharged to the outdoors without being high efficiency particulate air/HEPA filtered, subjected to thermal oxidation, or otherwise treated to prevent the release of viable organisms.
- Yes, No, N.A. Appendix K-V-O. The following personnel and operational practices shall be required:
- Yes, No, N.A. Appendix K-V-O-1. Personnel entry into the controlled area shall be through the entry area specified in Appendix K-V-N-1.
- Yes, No, N.A. Appendix K-V-O-2. Persons entering the controlled area shall exchange or cover their personal clothing with work garments such as jump suits, laboratory coats, pants and shirts, head cover, and shoes or shoe covers. On exit from the controlled area the work clothing may be stored in a locker separate from that used for personal clothing or discarded for laundering. Clothing shall be decontaminated before laundering.
- Yes, No, N.A. Appendix K-V-O-3. Entry into the controlled area during periods when work is in progress shall be restricted to those persons required to meet program or support needs. Prior to entry, all persons shall be informed of the operating practices, emergency procedures, and the nature of the work.

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| Yes, No, N.A. | Appendix K-V-O-4. Persons under 18 years of age shall not be permitted to enter the controlled area. |
| Yes, No, N.A. | Appendix K-V-O-5. The universal biosafety sign shall be posted on entry doors to the controlled area and all internal doors when any work involving the organism is in progress. This includes periods when decontamination procedures are in progress. The sign posted on the entry doors to the controlled area shall include a statement of agents in use and personnel authorized to enter the controlled area. |
| Yes, No, N.A. | Appendix K-V-O-6. The controlled area shall be kept neat and clean. |
| Yes, No, N.A. | Appendix K-V-O-7. Eating, drinking, smoking, and storage of food are prohibited in the controlled area. |
| Yes, No, N.A. | Appendix K-V-O-8. Animals and plants shall be excluded from the controlled area. |
| Yes, No, N.A. | Appendix K-V-O-9. An effective insect and rodent control program shall be maintained. |
| Yes, No, N.A. | Appendix K-V-O-10. Access doors to the controlled area shall be kept closed, except as necessary for access, while work is in progress. Service doors leading directly outdoors shall be sealed and locked while work is in progress. |
| Yes, No, N.A. | Appendix K-V-O-11. Persons shall wash their hands when exiting the controlled area. |
| Yes, No, N.A. | Appendix K-V-O-12. Persons working in the controlled area shall be trained in emergency procedures. |
| Yes, No, N.A. | Appendix K-V-O-13. Equipment and materials required for the management of accidents involving viable organisms containing recombinant DNA molecules shall be available in the controlled area. |
| Yes, No, N.A. | Appendix K-V-O-14. The controlled area shall be decontaminated in accordance with established procedures following spills or other accidental release of viable organisms containing recombinant DNA molecules. |